

Fluctuations, correlations and the estimation of concentrations inside cells

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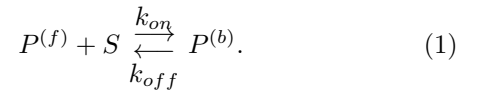
Information transmission in cells occurs quite accurately even when concentration changes are “read” by individual target molecules. In this Letter we study molecule number fluctuations when molecules diffuse and react. We show that, for immobile binding sites, fluctuations in the number of bound molecules are averaged out on a relatively fast timescale due to correlations. This result can explain the observed co-existence of highly fluctuating instantaneous transcriptional activities and of relatively stable protein concentrations shortly after the beginning of transcription. We also show that bound molecule numbers fluctuate with one or two characteristic timescales depending on the concentration of free molecules. This transition can explain changes in enzyme activity observed at the single molecule level.

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The transmission of information in cells usually involves changes in concentration that are “read” by target molecules. This occurs in a fluctuating environment. Yet cells respond quite reliably to various changes [1, 2]. The accuracy of the reading mechanism is key in the case of morphogens, molecules whose non-uniform distribution results in cell differentiation [3]. Most often this patterning process involves the binding of transcription factors to sites on DNA controlling the levels of proteins production. The relationship between the concentration of a protein and of the transcription factor that regulates its production depends on various binding processes. How faithful the spatial distribution of protein concentration reflects that of the transcription factor depends on how the concentration of the latter is read by the binding sites. This relationship has been studied during the early stages of development of *Drosophila melanogaster* embryos in great detail. The analysis of the variability of the concentrations of the protein Hunchback (Hb) and of the transcription factor Bicoid (Bcd) involved in its production shows that the resulting pattern is compatible with detecting [Bcd] with a 10% error [3]. Considering the random arrivals of individual Bcd molecules to a small neighborhood around a putative DNA binding site the calculations of [3] concluded that only after a long time ($\sim 2h$) compared to the developmental time of the embryo [Bcd] could be inferred with this level of precision. In [3] a spatial averaging between neighboring nuclei was invoked to reconcile this computation with the observations. As in [4], the time computed in [3] depends on the diffusion coefficient of Bcd for which there are estimates that differ by over an order of magnitude [5, 6]. These estimates have recently been shown to be compatible [7] if they are assumed to correspond to the two effective diffusion coefficients that describe the transport of molecules that diffuse and react [8]. In fact, Bcd, being a transcription factor, diffuses and reacts at least with putative binding sites on DNA. The analysis of [7] shows

that the two effective coefficients of Bcd can be very different between themselves. What is the diffusion coefficient that sets the limit for the precision with which [Bcd] can be read? Protein production, on the other hand, is determined by the number of bound rather than free transcription factor molecules. How do fluctuations in the number of bound and free molecules relate to each other? In this paper we address these two issues. Building on previous works on the analysis of optical experiments when molecules diffuse and react [9–11] we derive expressions for the relative error with which the concentrations involved can be estimated as a function of the observation time and apply it to interpret recent results on the variability of mRNA production [12, 13] and on enzyme turnovers at the single molecule level [14]. In particular, we show that the interaction with immobile binding sites introduces correlations that reduce the variance of the bound molecules number and that the observation time that is needed to estimate the concentration of these molecules with a given accuracy depends on a relatively fast correlation time.

In this Letter we consider a system of particles (*e.g.*, transcription factors), $P^{(f)}$, that diffuse with (free) coefficient, D_f , and react with binding sites, S , according to [8, 10, 11]:



We assume that the binding sites diffuse with coefficient $D_S \ll D_f$ (D_S can be zero) and that the mass of S is so large that the free coefficient of $P^{(b)}$ is D_S too. We consider a total volume, V_T , over which the molecules diffuse and the concentrations, $[P^{(f)}]$, $[P^{(b)}]$, $[S]$, are approximately constant, uniform and in equilibrium among themselves ($[P^{(f)}][S] = K_D[P^{(b)}]$), and an observation volume, V_{obs} , where we count the number of molecules of the three species, $N^{(f)}$, $N^{(b)}$ and $N^{(S)}$, every time step, dt . These are the stochastic variables

of the problem which means satisfy $\langle N^{(f)} \rangle = [P^{(f)}]V_{obs}$, $\langle N^{(b)} \rangle = [P^{(b)}]V_{obs}$ and $\langle N^{(S)} \rangle = [S]V_{obs}$ if $D_s \neq 0$. If $D_S = 0$ and $V_{obs} \ll V_T$, there could be a local equilibrium in V_{obs} slightly different from the one in V_T that depends on the (fixed) total number of binding sites in V_{obs} , $N_{ST} \equiv N^{(b)} + N^{(S)}$. The aim is to determine the difference between the mean and the average, $\overline{N}^{(s)}(T_{obs}) = \frac{\sum_{\ell} N_{\ell}^{(s)}}{n}$ of each stochastic variable ($s = f, b, S$) after an observation time, $T_{obs} = ndt$ (i.e., from a sequence $\{N_{\ell}^{(s)} \equiv N^{(s)}(t_{\ell})\}_{\ell=0}^{n-1}$). This difference will allow us to estimate the time that is needed to derive $\langle N^{(s)} \rangle$ with a given accuracy from counting molecules in V_{obs} . The (mean) square difference between $\overline{N}^{(s)}(T_{obs})$ and $\langle N^{(s)} \rangle$ is the variance of the average,

$$\begin{aligned} \text{var} \left(\overline{N}^{(s)}(T_{obs}) \right) &= \text{var} \left(\frac{1}{n} \sum_{\ell=0}^{n-1} N_{\ell}^{(s)} \right) \\ &= \frac{1}{n^2} \left\langle \sum_{\ell,k=0}^{n-1} \left(N_{\ell}^{(s)} - \langle N^{(s)} \rangle \right) \left(N_k^{(s)} - \langle N^{(s)} \rangle \right) \right\rangle, \quad (2) \end{aligned}$$

which is related to the autocorrelation function (ACF) of the particle number fluctuations. Using the normalization of FCS experiments, the ACF for species s is given by $G^{(s)}(\tau)/\langle N^{(s)} \rangle^2$ with $G^{(s)}(\tau = jdt) = \lim_{n \rightarrow \infty} \sum_{\ell=0}^{n-1} \left(N_{\ell}^{(s)} - \langle N^{(s)} \rangle \right) \left(N_{\ell+j}^{(s)} - \langle N^{(s)} \rangle \right) / n$. For systems with one species that diffuses with coefficient, D , and a Gaussian V_{obs} of width, w_r , it is [15]:

$$G(\tau) = \frac{\text{var}(N)}{\left(1 + \frac{\tau}{\tau_D}\right)^{3/2}}, \quad (3)$$

with $\tau_D = w_r^2/4D$, N the number of molecules in V_{obs} and $\text{var}(N) = \langle N \rangle$. $G(\tau)$ in Eq. (3) is relatively flat with $G(\tau) \approx \text{var}(N)$ for $\tau \leq \tau_D$ and $G(\tau) \approx 0$ otherwise. We then approximate $\frac{1}{n} \sum_{\ell=0}^{n-1} (N_{\ell} - \langle N \rangle) (N_k - \langle N \rangle) \approx \text{var}(N)$ for $|k - \ell| \leq \tau_D/dt$ and assume it is negligible otherwise. If $n \geq \tau_D/dt$ we then obtain:

$$\text{var} \left(\overline{N}(T_{obs}) \right) \approx \frac{1}{n} \sum_{k=-\tau_D/dt}^{\tau_D/dt} \text{var}(N) \approx \frac{2\tau_D}{T_{obs}} \text{var}(N), \quad (4)$$

with the last approximation being valid for $\tau_D \gg dt$. If $n \leq \tau_D/dt$ the same formula is obtained but with 1 instead of τ_D/T_{obs} . Replacing $\tau_D = w_r^2/4D$ in Eq. (4) we obtain a similar error of the average as the one considered in [3, 4]. Eq. (4) implies that the relative error, $\Delta_r(\overline{N}) \equiv (\text{var}(\overline{N}(T_{obs})))^{1/2}/\langle N \rangle$ decreases with the correlation time and $\text{var}(N)$. The necessary time to obtain an estimate with relative error α , on the other hand, is $T_{obs}(\alpha) \sim \tau_D \text{var}(N)/(\alpha \langle N \rangle)^2$.

When the reaction-diffusion system of species that corresponds to Eq. (1) is considered there is more than one correlation time. Working in Fourier space as in [15] we

obtain 3 branches of eigenvalues, λ_i , that rule the fluctuations dynamics. λ_1 always corresponds to the free diffusion time of the binding sites, $\tau_S \equiv w_r^2/4D_S$ [10, 11]. λ_2 and λ_3 have a clear meaning in the fast diffusion ($\tau_f \equiv w_r^2/(4D_f) \ll \tau_r \equiv 1/(k_{off}(1 + [P^{(f)}]/K_D + [S]/K_D))$), and in the fast reaction ($\tau_r \ll \tau_f$) limits. In both limits the eigenvalues can be written as $-\nu_i - D_i q^2$, with q the variable conjugate to position in Fourier space. They contribute to $G^{(s)}(\tau)$ with an additive term of the form:

$$G_i^{(s)}(\tau) = \frac{G_{oi}^{(s)}}{\left(1 + \frac{\tau}{\tau_{Di}}\right)^{3/2}} e^{-\nu_i \tau}, \quad s = f, b, S, \quad (5)$$

where $\tau_{Di} = w_r^2/4D_i$ for a Gaussian V_{obs} and the weights, $G_{oi}^{(s)}$, are linear combinations of the covariances between the stochastic variables that satisfy $G_o^{(s)} \equiv \sum_i G_{oi}^{(s)} = \text{var}(N^{(s)})$ [9, 16]. In both limits it is $\nu_i = 0$ if $D_i \neq 0$ and viceversa. Thus, there is a single correlation time, τ_i , associated to each eigenvalue and to each term, $G_i^{(s)}(\tau)$, which is diffusive (if $D_i \neq 0$) or determined by the reactions only (if $D_i = 0$). Ordering the times so that $\tau_1 \geq \tau_2 \geq \tau_3$, it is $\tau_1 = \tau_S$ always, unless $D_S = 0$ in which case it disappears from the ACF, as explained later. In the fast diffusion limit, it is $\tau_3 = w_r^2/4D_f$ while $\tau_2 = w_r^2/4D_S$ (if $D_S \neq 0$) or $\tau_2 = \tau_{off} \equiv k_{off}^{-1} \langle N^{(S)} \rangle / N_{ST}$ (if $D_S = 0$). In the fast reaction limit it is $\tau_3 = \tau_r$ while $\tau_2 = \tau_{coll} \equiv w_r^2/4D_{coll}$ with D_{coll} a (reaction-dependent) “effective” diffusion coefficient: $D_{coll} = (D_f + \frac{[S]^2}{K_D[S_T]} D_S) / (1 + \frac{[S]^2}{K_D[S_T]})$ where $[S_T] = [P^{(b)}] + [S]$ [8, 11]. τ_{coll} can be of the order of τ_f even if a relatively large fraction of the particles is bound [8]. As for Eq. (3), we assume that $G_i^{(s)}(\tau) \approx G_{oi}^{(s)}$ if $|\tau| \leq \tau_i$ and negligible otherwise. Applying this approximation to compute $\text{var}(\overline{N}^{(s)})$ and assuming $\tau_i \gg dt$ and $n \geq \tau_i/dt$ we obtain:

$$(\Delta_r \overline{N}^{(s)})^2(T_{obs}) = \frac{\text{var}(\overline{N}^{(s)})}{\langle N^{(s)} \rangle^2} \approx \frac{\text{var}(N^{(s)})}{\langle N^{(s)} \rangle^2} \sum_{i=1}^3 W_i^{(s)} \frac{2\tau_i}{T_{obs}}, \quad (6)$$

where $W_i^{(s)} \equiv G_{oi}^{(s)}/G_o^{(s)}$ (so that $\sum_i W_i^{(s)} = 1$). Also in this case the relative errors increase with the correlation times and $\text{var}(N^{(s)})$ and decrease when $\langle N^{(s)} \rangle$ increases. As before, the ratio τ_i/T_{obs} has to be replaced by 1 in Eq. (6) if $T_{obs} \leq \tau_i$.

How relevant each correlation time is for a species, s , depends on the relative weight, $W_i^{(s)}$. If $D_S \neq 0$, we assume as usual [15] that $\text{cov}(N_{\ell}^{(s)}, N_{\ell}^{(v)}) = \langle N^{(s)} \rangle \delta_{s,v}$, with $s, v = f, b, S$ [16]. If $D_S = 0$, given that $N^{(b)} + N^{(S)}$ is fixed, we assume that $N^{(b)}$ and $N^{(S)}$ are binomial so that $\text{var}(N^{(S)}) = \text{var}(N^{(b)}) = \langle N^{(b)} \rangle \langle N^{(S)} \rangle / N_{ST}$, and $\text{cov}(N_{\ell}^{(b)}, N_{\ell}^{(S)}) = -\text{var}(N^{(b)})$. The weights for $s = f$ do not depend on this assumption, but those of $s = b, S$, do. Namely, $W_1^{(f)} = 0$ always while $W_1^{(b)} = 0$ if $D_S = 0$ due

to the correlations between $N^{(b)}$ and $N^{(s)}$ but it is finite ($= f_b \equiv \langle N^{(b)} \rangle / N_{ST}$) even if D_S is arbitrarily small [16]. The finite change in $W_1^{(b)}$ is reflected in the ACF as illustrated in Figs. 1 (a,b) where we show $G^{(b)}(\tau) / \langle N^{(b)} \rangle$ computed from numerically generated time-series (symbols) and using Eq. (5) (lines) with the analytic weights of the fast diffusion (a) and the fast reaction (b) limits. To generate the series we performed stochastic simulations of particles that diffuse with $D_f = 19\mu\text{m}^2/\text{s}$ and react according to Eq. (1) with $K_D = 0.192\mu\text{M}$ using a Gillespie-like scheme [10, 17]. $G_o^{(b)}$ and $\text{var}(N^{(b)})$ are smaller for the cases with $D_S = 0$ than for the corresponding ones with $D_S \neq 0$ although $\langle N^{(b)} \rangle$ is the same in each subfigure. The difference is noticeable because $f_b \approx 0.92$. Fig. 1 (a) also illustrates the change of the relevant timescales that is observed in the fast diffusion limit when $D_S = 0$. Namely, in this limit, fluctuations in $N^{(b)}$ depend on τ_S if $D_S \neq 0$ and on τ_{off} if $D_S = 0$. In this figure we have superimposed the results obtained for various values of k_{off} . Changes of the ACF with k_{off} are unobservable for $D_S \neq 0$ while they are noticeable for $D_S = 0$. The change in the relevant timescale in the fast reaction limit is illustrated in Fig. 1 (c). There we observe that $\bar{N}^{(b)}$ approaches its expected value, $\langle N^{(b)} \rangle$, faster if $D_S = 0$ than if it is $D_S = 0.2\mu\text{m}^2\text{s}^{-1}$ since the slowest timescale of the latter, τ_S , is absent in the former.

We compare now the necessary time, $T_{obs}(\alpha)$, to estimate $\bar{N}^{(f)}$ and $\bar{N}^{(b)}$ with a relative error, α , when $D_S = 0$. Identifying $\bar{N}^{(f)}$ and $\bar{N}^{(b)}$ with the free and the DNA-bound transcription factor molecules we can apply this comparison to study the accuracy of transcription, given that the resulting protein concentration (accumulated up to T_{obs}) depends on $\sum_{\ell=1}^{T_{obs}/dt} N_{\ell}^{(b)} = T_{obs} \bar{N}^{(b)}(T_{obs})$. Eq. (6) implies that $T_{obs}(\alpha)$ scales linearly with $\text{var}(N^{(s)}) / \langle N^{(s)} \rangle^2$. We have discussed that, if $D_S = 0$, $\text{var}(N^{(b)}) = \langle N^{(b)} \rangle (1 - f_b)$ while fluctuations in $N^{(f)}$ follow a Poisson distribution. Regardless of the correlation times, then $\bar{N}^{(b)}$ can be within a few percent of its expected value if $D_S = 0$ and $f_b \approx 1$. An interesting situation can be found in the fast reaction limit. In this limit $\Delta_r(\bar{N}^{(f)})$ and $\Delta_r(\bar{N}^{(b)})$ depend on τ_{coll} and τ_r with weights $W_2^{(f)} = W_3^{(b)} = (1 + \beta)^{-1}$ and $W_3^{(f)} = W_2^{(b)} = 1 - W_2^{(f)}$ where $\beta = \langle N^{(s)} \rangle^2 / (K_D N_{ST} V_{obs})$. Regardless of f_b , β can be larger or smaller than 1 depending on $\langle N^{(s)} \rangle / \langle N^{(f)} \rangle$. If $\beta < 1$, it is $W_2^{(f)} = W_3^{(b)} > W_3^{(f)} = W_2^{(b)} = 1 - W_2^{(f)}$ so that $\bar{N}^{(b)}$ approaches its expected value over a faster timescale than $\bar{N}^{(f)}$. Furthermore, even if $f_b \sim 1$, the time, τ_{coll} , can be of the same order as the particles free diffusion time, τ_f . This implies that there are parameters for which $f_b \sim 1$, so that $\text{var}(N^{(b)})$ is sensitively reduced with respect to the Poisson case and, at the same time, the effective diffusion coefficient, $D_{coll} \sim D_f$, so that the slowest convergence time of

$(\bar{N}^{(b,f)})$ is of the same order as τ_f . This combination of parameters is not just a speculation. Analyzing the FCS experiments of [6] on the diffusion of Bcd in *Drosophila melanogaster* embryos under the assumption that Bcd diffuses and reacts following Eq. (1) and that the fast reaction limit holds we found $\tau_{coll} \sim 0.7\tau_f$, $\beta \sim 0.4$ and $f_b \sim 0.92 - 0.98$ [7]. We illustrate in Fig. 2 (a,b) how, for the same combination of times and fraction of bound sites as those deduced in [7], $\Delta_r(\bar{N}^{(b)}) < \Delta_r(\bar{N}^{(f)})$ for any T_{obs} by a factor that cannot be accounted for by the difference between $\langle N^{(b)} \rangle$ and $\langle N^{(f)} \rangle$. Namely, $\langle N^{(f)} \rangle \sim 2200$, $\langle N^{(b)} \rangle \sim 22000$ in this figure so that $\Delta_r(\bar{N}^{(b)}) / \Delta_r(\bar{N}^{(f)}) \sim 0.17 (\langle N^{(f)} \rangle / \langle N^{(b)} \rangle)^{1/2}$ for any T_{obs} . This figure also shows that Eq. (6) provides relatively good estimates of $\Delta_r(\bar{N}^{(s)})$. It is important to note that $\Delta_r(\bar{N}^{(b)})$ depends on the fraction of bound binding sites in V_{obs} , which is a stochastic variable that we approximate by $\langle N^{(b)} \rangle / N_{ST}$ in Eq. (6). When many reactions occur during the diffusion timescale (*i.e.*, in the case of Figs. 2 (a,b)), most of the time the molecule numbers are in equilibrium between themselves and f_b is close to its expected value. Something different can occur in systems with small V_{obs} where the fast diffusion limit holds. This is the situation that a binding site “encounters” when “trying” to infer the concentration of its ligand as considered in [3]. A rough way to treat the stochasticity of f_b is to assume it has an associated error and propagate it in $\Delta_r(\bar{N}^{(b)})$. In this way we obtain $\Delta f_b = (\text{var}(N^{(f)}))^{1/2} K_D V_{obs} / (K_D V_{obs} + \langle N^{(f)} \rangle)$ and, using $\tau_{off} = (1 - f_b) / k_{off}$,

$$\left(\Delta_r(\bar{N}^{(b)}) \right)^2 \approx \frac{(1 - f_b)}{\langle N^{(b)} \rangle} \frac{\tau_{off}}{T_{obs}} \left(1 + 2 \sqrt{\frac{\tau_f}{T_{obs} \langle N_f \rangle}} \right), \quad (7)$$

where we have assumed that the fast diffusion holds and $T_{obs} \geq \tau_i$ ($i = off, f$). If $T_{obs} < \tau_i$, the ratio, τ_i / T_{obs} must be replaced by 1. Fig. 2 (c) illustrates how $\Delta_r(\bar{N}^{(b)})$ varies with T_{obs} when this effect is relevant. It is the equivalent of Fig. 2 (b) for a system in the fast diffusion limit and where Eq. (7) is also plotted with dashed curves. We observe that Eq. (7) captures well the decay of $\Delta_r(\bar{N}^{(b)})$ with T_{obs} which occurs faster than if the convergence of f_b is not considered. Eq. (7) can be used to interpret recent observations of transcriptional regulation *in vivo* in *Drosophila melanogaster* embryos [12, 13]. These studies show that the instantaneous production of the Hb mRNA varies up to 50% between loci of transcription while the resulting cytoplasmic mRNA and protein concentrations in a volume embracing a nucleus fluctuate by less than 10%. The protein, Hb, is long-lived and accumulates with time, so that time averaging can be responsible for smoothing the instantaneous fluctuations out. The increase in precision, however, cannot be explained by time averaging so that the occurrence of some spatial averaging was invoked in [12]. The diffusion of

the free transcription factors (our P_f) between loci and its effect on the convergence of f_b as included in Eq. (7) could be the mechanism that underlies the smoothing out of fluctuations in $\overline{N}^{(b)}$ and thus, on the number of Hb mRNA molecules, on a faster timescale than the one prescribed by time averaging (Eq. (6)).

In this Letter we have presented results obtained in two opposite limits. From their differences we can infer the types of situations that may be found in between. The convergence times of the average number of free and bound particles are different depending on the limit. Various quantities can be varied to change the ratio, τ_f/τ_r , that rules the transition between limits. As expected, τ_f , decreases with increasing D_f and τ_r with increasing k_{off} [10]. τ_f also decreases with V_{obs} and τ_r with increasing concentrations. Thus, by considering a small or a large V_{obs} not only the fluctuation sizes change due to the different numbers of particles but also the correlation times change with their corresponding effect on the errors in molecule number estimates. This has implications in morphogenesis. It can also be related to the changes observed in single enzyme activities [14]. The first step of a Michaelis-Menten scheme in which a substrate, $P^{(f)}$, binds to an enzyme, S , and is then transformed into a product at a rate proportional to $[P^{(b)}]$ is given by Eq. (1). The observation of these reactions at the single molecule level showed a distribution of waiting times between individual turnovers that was mono-exponential at low $[P^{(f)}]$ and was characterized by several timescales at high $[P^{(f)}]$ [14]. Based on the results presented in this Letter we can interpret this change in terms of a transition from the fast diffusion to the fast reaction limit as $[P^{(f)}]$ increases. This involves a change in the timescales of the $N^{(b)}$ fluctuations: from the single non-diffusive timescale, τ_{off} , to two timescales, τ_r and τ_{coll} (with the additional timescale, τ_f , if the effect of Fig. 2 (c) is included). The appearance of a diffusive timescale as the fast reaction limit is approached can also underlie the change to a “broad” dwell time distribution observed in [14].

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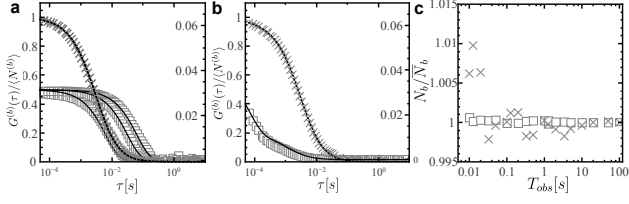


FIG. 1. (a) $G^{(b)}(\tau)/\langle N^{(b)} \rangle$ for a system of particles that diffuse and react with immobile (\square) or mobile (\times) sites in the limit of fast diffusion for $k_{off} = 0.5s^{-1}$, $1s^{-1}$, $5s^{-1}$. Variations of $G^{(b)}(\tau)/\langle N^{(b)} \rangle$ with k_{off} are apparent for $D_S = 0$. (b) $G^{(b)}(\tau)/\langle N^{(b)} \rangle$ as in (a) but for other concentrations, $k_{off} = 400s^{-1}$ so that the fast reaction limit holds and $D_S = 0$ (\square) or $5\mu m^2 s^{-1}$ (\times). In (a) and (b), the curves are the theoretical predictions for $D_S \neq 0$ (dashed, scale at left) and $D_S = 0$ (solid, scale at right). (c) $N^{(b)}(T_{obs})/\langle N^{(b)} \rangle$ vs. T_{obs} for the same parameters as in (b) and $D_S = 0$ (\square) or $0.2\mu m^2 s^{-1}$ (\times).

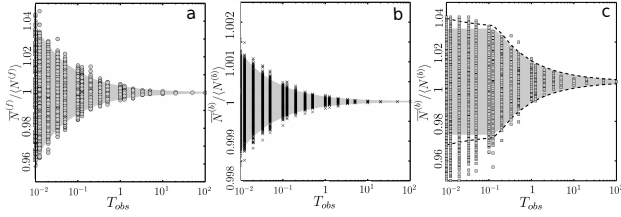


FIG. 2. Normalized average numbers of free, $N^{(f)}$ (a), and bound, $N^{(b)}$ (b, c), particles obtained from stochastic simulations (\square) of reaction-diffusion systems with $D_S = 0$ and relative errors, $\Delta_r(\overline{N}^{(f,b)})$, given by Eq. (6) (gray shaded areas) as functions of T_{obs} . In (a,b) the parameters are the same as in Fig. 1 (c) and in (c) as in Figs. 1 (a,b). In (c) the error given by Eq. (7) is also shown (dashed curves).

Supporting Information for Fluctuations, correlations and the estimation of concentrations inside cells

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THE SYSTEM

We consider a system [1–3] composed of molecules $P^{(f)}$, that diffuse with free coefficient, D_f , and react with binding sites, S , according to:



where the rates of binding and unbinding define the dissociation constant, $K_D = \frac{k_{off}}{k_{on}}$. We assume that the binding sites are much larger than the particles so that $P^{(b)}$ and S diffuse with $D_S \ll D_f$. We consider a total volume, V_T , over which the molecules diffuse and the concentrations, $[P^{(f)}]$, $[P^{(b)}]$, $[S]$, are approximately constant, uniform and in equilibrium among themselves ($[P^{(f)}][S] = K_D[P^{(b)}]$), and an observation volume, V_{obs} , where we count the number of molecules, $N^{(f)}$, $N^{(b)}$ and $N^{(S)}$, every time step, dt . We assume that the mean, $\langle N^{(f)} \rangle$, satisfies $\langle N^{(f)} \rangle = [P^{(f)}]V_{obs}$. The relations $\langle N^{(b)} \rangle = [P^{(b)}]V_{obs}$ and $\langle N^{(S)} \rangle = [S]V_{obs}$ (which, due to the equilibrium condition imply that $\langle N^{(b)} \rangle = (\langle N^{(b)} \rangle + \langle N^{(S)} \rangle)[P^{(f)}]/([P^{(f)}] + K_D)$), are assumed to hold if $D_S \neq 0$. If $D_S = 0$ we assume that $\langle N^{(b)} \rangle = N_{ST}[P^{(f)}]/([P^{(f)}] + K_D)$ and $\langle N^{(S)} \rangle = N_{ST} - \langle N^{(b)} \rangle$, with $N_{ST} \equiv N^{(b)} + N^{(S)}$, the (fixed) total number of binding sites in V_{obs} .

AUTOCORRELATION FUNCTION. ANALYTIC CALCULATIONS

We consider the sequences, $\{N_\ell^{(s)} \equiv N^{(s)}(t_\ell)\}_{\ell=0}^{n-1}$, obtained after an observation time, $T_{obs} = ndt$ for the three species, $s = f, b, S$, and compute $G^{(s)}(\tau = jdt) = \lim_{n \rightarrow \infty} \sum_{\ell=0}^{n-1} (N_\ell^{(s)} - \langle N^{(s)} \rangle) (N_{\ell+j}^{(s)} - \langle N^{(s)} \rangle) / n$ for each of them. Dividing each of these functions by $\langle N^{(s)} \rangle^2$ we obtain the autocorrelation function (ACF) as defined in Fluorescence Correlation Spectroscopy (FCS) experiments. In this case, it is the ACF of the molecule number fluctuations of each species in V_{obs} . This computation is easy to perform in the case of numerical simulations. For the analytic calculations it is simpler to work as in the case of FCS experiments. Namely, instead of adding all the particles of species (s) in V_{obs} to compute $N^{(s)}$, we add all the particles of species (s) in V_T but with a Gaussian weight: $N^{(s)} = \int_{V_T} d^3\vec{r} I(\vec{r}) c^{(s)}$ where $I(\vec{r}) = \exp\left(-2\frac{r^2}{w_r^2}\right)$, $r = |\vec{r}|$, w_r is the waist of the Gaussian and $c^{(s)} = \sum_{i_s} \delta(\vec{r} - \vec{r}_{i_s}(t))$ with the sum running over all the molecules of species (s) and $\vec{r}_{i_s}(t)$ the location of each of them at time t . In this way, it is $V_{obs} = \int d^3\vec{r} I(\vec{r}) = (\pi/2)^{3/2} w_r^3$ and:

$$G^{(s)}(\tau) = \langle \delta N^{(s)}(t) \delta N^{(s)}(t + \tau) \rangle = \frac{1}{T_{obs}} \int d\vec{r} \int d\vec{r}' \int_0^{T_{obs}} dt I(\vec{r}) I(\vec{r}') \delta c^{(s)}(\vec{r}, t) \delta c^{(s)}(\vec{r}', t + \tau), \quad (2)$$

where $\delta c^{(s)} \equiv c^{(s)}(\vec{r}, t) - \langle N^{(s)} \rangle / V_{obs}$. As done in [4, 5], for the analytic computation of $G^{(s)}(\tau)$ we calculate the differences, $\delta c^{(s)}$, for the 3 species of the system, as the solution of the reaction-diffusion equations linearized around equilibrium. This solution can be written in Fourier space in terms of the (branches of) eigenvalues and eigenvectors of the linear system so that $G^{(s)}$ can be expressed as:

$$G^{(s)}(\tau) = \int d\vec{q} |\hat{I}(\vec{q})|^2 \sum_m X_j^{(m)} \exp(\lambda^{(m)} \tau) (X^{-1} \sigma^2)_j^{(m)} \quad (3)$$

where the subscript, j , refers to the species ($j = 1$ for $s = S$, $j = 2$ for $s = f$ and $j = 3$ for $s = b$) and the index, (m) , labels the eigenvalues, $\hat{I}(\vec{q})$ is the Fourier transform of $I(\vec{r})$ and \vec{q} is the conjugate variable of \vec{r} , X is the matrix of eigenvectors, $\lambda^{(m)}$ is the m -th eigenvalue and σ^2 is the matrix of initial correlations between the species, $\sigma_{ij}^2 = \langle \delta N^{(s)}(t) \delta N^{(s')}(t) \rangle 2^{3/2} / V_{obs}$ with i, j the indices corresponding to species s and s' , respectively. In [5] it is

assumed that the initial correlations satisfy $\langle \delta c^{(s)}(\vec{r}, t) \delta c^{(s')}(\vec{r}', t) \rangle = \langle c^{(s)} \rangle \delta_{ij} \delta(\vec{r} - \vec{r}')$ with δ_{ij} the Kronecker delta. This assumption implies that the correlations are spatially short-ranged, that the number of molecules of different species in V_{obs} are uncorrelated and that, for each species, they are Poisson distributed. In deriving Eq. (3) we have also assumed that the initial correlations are short ranged $\langle \delta c^{(s)}(\vec{r}, t) \delta c^{(s')}(\vec{r}', t) \rangle \propto \delta(\vec{r} - \vec{r}')$ but, as in [4], we have relaxed here the assumption on the Poisson distribution. Namely, the assumption that the molecule numbers follow a Poisson distribution is valid if the species diffuses, but in the case with $D_S = 0$, $N^{(b)}$ and $N^{(S)}$ are correlated. In such a case, we assume that they follow a binomial distribution (with $N^{(S)} + N^{(b)} = N_{ST}$ constant), so that $\langle \delta c^{(S)}(\vec{r}, t) \delta c^{(b)}(\vec{r}', t) \rangle = -\langle \delta c^{(b)}(\vec{r}, t) \delta c^{(b)}(\vec{r}', t) \rangle = -\langle N^{(b)} \rangle (1 - f_b) \delta(\vec{r} - \vec{r}') 2^{3/2} / V_{obs}$, where $f_b = \langle N^{(b)} \rangle / N_{ST}$ is the fraction of bound molecules in V_{obs} [4]. Including the initial correlation matrix in Eq. (3) provides a unifying notation which embraces all the situations analyzed in our Letter. Eq. (3) generally does not have an analytical solution, but in [3] it was shown that there exist two limits in which the three eigenvalue branches of the system can be expressed as $\lambda_i = -\nu_i - D_i q^2$. These limits are the *fast reaction* limit, (*fr*), in which reactions take place on a much faster time scale than diffusion in V_{obs} , and the *fast diffusion* limit, (*fd*), in which the opposite relationship between the time scales holds. In both these limits it is:

$$G^{(s)}(\tau) = \sum_{i=1}^3 G_i^{(s)}(\tau) = \sum_{i=1}^3 \frac{G_{oi}^{(s)}}{\left(1 + \frac{\tau}{\tau_{Di}}\right)^{3/2}} e^{-\nu_i \tau}, \quad (4)$$

where $\tau_{Di} = w_r^2 / 4D_i$ and the weights, $G_{oi}^{(s)}$, are linear combinations of the covariances between the stochastic variables that satisfy $G_o^{(s)} \equiv \sum_i G_{oi}^{(s)} = \text{var}(N^{(s)})$. Deriving from Eq. (3) the weights, $G_{oi}^{(f)}$ and $G_{oi}^{(b)}$, in both limits as done in [3] but including the matrix of initial correlations as done in [4] we obtain the expressions listed in table I. In this table we show the values obtained when $D_S = 0$ or $D_S \neq 0$ in the fast diffusion and the fast reaction limits. From these values, the relative weights, $W_i^{(s)} = G_{oi}^{(s)} / G_o^{(s)}$, $s = f, S$, can be derived.

NUMERICAL SIMULATIONS

We performed stochastic numerical simulations of the reaction-diffusion system considered with a Gillespie-like algorithm [3, 6]. To compute $N^{(s)}(t)$ we either worked as with the analytic computations, namely, we computed $N^{(s)} = \sum_i I(\vec{r}_i) N^{(s)}(\vec{r}_i, t)$ where $N^{(s)}(\vec{r}_i, t)$ is the number of molecules of species s in a volume of size, $d\vec{r}_i = 1.25 \times 10^{-4} \mu m^3$, centered at \vec{r}_i , or we counted all those inside a cube of size $0.016 \mu m^3$ with the same weight (*i.e.*, we computed $N^{(s)} = \sum_i I(\vec{r}_i) N^{(s)}(\vec{r}_i, t)$ with $I = 1$ for $|x| \leq 0.25 \mu m$, $|y| \leq 0.25 \mu m$, $|z| \leq 0.25 \mu m$, and $I = 0$ otherwise). For the system parameters we used the ones listed in the Table which were derived from an analysis [7] of FCS experiments performed in *Drosophila melanogaster* to estimate the diffusion coefficient of the protein Bicoid [8].

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		$G_1(\tau)$				$G_2(\tau)$				$G_3(\tau)$			
		$G_{o1}^{(f)}$	$G_{o1}^{(b)}$	τ_{D1}	ν_1	$G_{o2}^{(f)}$	$G_{o2}^{(b)}$	τ_{D2}	ν_2	$G_{o3}^{(f)}$	$G_{o3}^{(b)}$	τ_{D3}	ν_3
$D_S \neq 0$	<i>fr</i>	0	$f_b \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_S}$	0	$\frac{1}{(1+\beta)} \langle N^{(f)} \rangle$	$\frac{(1-f_b)\beta}{(1+\beta)} \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_{coll}}$	0	$\frac{\beta}{(1+\beta)} \langle N^{(f)} \rangle$	$\frac{(1-f_b)}{(1+\beta)} \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_{ef2}}$	$\frac{k_{off}(1+\beta)}{1-f_b}$
	<i>fd</i>	0	$f_b \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_S}$	0	0	$(1-f_b) \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_S}$	$\frac{k_{off}(1+\beta)}{1-f_b}$	$\langle N^{(f)} \rangle$	0	$\frac{w_r^2}{4D_f}$	0
$D_S = 0$	<i>fr</i>	0	0	$\frac{w_r^2}{4D_S}$	0	$\frac{1}{(1+\beta)} \langle N^{(f)} \rangle$	$\frac{(1-f_b)\beta}{(1+\beta)} \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_{coll}}$	0	$\frac{\beta}{(1+\beta)} \langle N^{(f)} \rangle$	$\frac{(1-f_b)}{(1+\beta)} \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_{ef2}}$	$\frac{k_{off}(1+\beta)}{1-f_b}$
	<i>fd</i>	0	0	$\frac{w_r^2}{4D_S}$	0	0	$(1-f_b) \langle N^{(b)} \rangle$	$\frac{w_r^2}{4D_S}$	$\frac{k_{off}}{1-f_b}$	$\langle N^{(f)} \rangle$	0	$\frac{w_r^2}{4D_f}$	0

TABLE I: Expressions for the weights, $G_{oi}^{(f)}$ and $G_{oi}^{(b)}$, in the cases with $D_S = 0$ or $D_S \neq 0$ and in the fast diffusion (*fd*) or the fast reaction (*fr*) limits, where $f_b = \frac{\langle N^{(b)} \rangle}{N_{ST}}$, $D_{coll} = \frac{D_f + \beta D_S}{1+\beta}$, $D_{ef2} = \frac{\beta D_f + D_S}{1+\beta}$ and $\beta = \frac{\langle N^{(S)} \rangle^2}{K_D N_{ST} V_{obs}}$. In the Letter we assume that, in the fast reaction limit, ν_3 is so large that the corresponding weight is almost negligible when the slow diffusion timescale associated to D_{ef2} is reached so that it is not necessary to include the latter in the description. The opposite situation is assumed to hold in the fast diffusion limit between the timescales associated to D_S and ν_2 .

	Fig. 1(a)	Fig. 1(b-c)	Fig. 2(a-b)	Fig. 2(c)
D_S	$[0 - 5] \mu m^2 s^{-1}$	$[0 - 5] \mu m^2 s^{-1}$	$0 \mu m^2 s^{-1}$	$0 \mu m^2 s^{-1}$
D_f	$19 \mu m^2 s^{-1}$	$19 \mu m^2 s^{-1}$	$19 \mu m^2 s^{-1}$	$19 \mu m^2 s^{-1}$
k_{off}	$[0.5 - 1 - 5] s^{-1}$	$400 s^{-1}$	$400 s^{-1}$	$0.5 s^{-1}$
K_D	$1.92 nM$	$0.2496 \mu M$	$0.2496 \mu M$	$1.92 nM$
$[S]$	$22.1 nM$	$2.87 \mu M$	$2.87 \mu M$	$22.1 nM$
$[P^{(f)}]$	$59.1 nM$	$7.68 \mu M$	$7.68 \mu M$	$59.1 nM$
$[P^{(b)}]$	$679.3 nM$	$88.31 \mu M$	$88.31 \mu M$	$679.3 nM$
T_{obs}	$100 s$	$100 s$	$100 s$	$100 s$
$I(\vec{r})$	Gaussian	Gaussian	Cubic	Cubic
V_{obs}	$0.053 \mu m^3$	$0.053 \mu m^3$	$0.016 \mu m^3$	$0.016 \mu m^3$

TABLE II: Parameters of the simulations.